CHAPTER 4

INITIAL ASSESSMENT OF DATA

4.1 INTRODUCTION[1]

Following data gathering, testing and data evaluation as described in chapters 2 and 3, the hazards of a given chemical need to be assessed and described in the SIDS Initial Assessment Report (SIAR) or the Initial Targeted Assessment Report (ITAR). Guidance on the structure and content of the SIAR and ITAR can be found in Chapter 5. Summary conclusions on hazard endpoints will be reported in the SIDS Initial Assessment Profile (SIAP) or Initial Targeted Assessment Profile (ITAP), fir which guidance is provided in Chapter 6.

There are currently two sections in Chapter 4 of the *Manual for the Assessment of Chemicals* that give guidance on how to assess the hazards of a chemical substance:

- **Section 4.2**: Guidance for the Initial Assessment of Aquatic Effects, describes how to use all available data on toxicity to aquatic organisms, physical-chemical properties and environmental fate to determine and conclude on the level of hazard to the aquatic environment. The data presented should allow end users of the assessment to estimate a concentration where no unacceptable adverse effects on the aquatic ecosystem are expected (i.e. Predicted No Effect Concentration, PNEC). At present, no guidance is yet available on the assessment of effects towards terrestrial and benthic organisms.
- **Section 4.3**: Guidance for the Initial Assessment of Health Effects, provides guidance on how to assess the available results on acute toxicity, irritation, sensitisation, repeated dose toxicity, genetic toxicity and reproduction/developmental toxicity. It also provides some guidance on whether available test results should be considered adequate to make a conclusion regarding an endpoint. Genetic toxicity is an example where such consideration is particularly needed, given the number of test guidelines and the possibility of having *in vitro* and *in vivo* data. .

Although no actual hazard classification is performed within the OECD Programme on the Cooperative Assessment of Chemicals, the terminology found in the classification criteria should be used. Summary conclusions on hazard endpoints should be as clearly formulated as possible to enable classification according to the GHS if needed.

The OECD Cooperative Chemicals Assessment Programme will strive to remain at the forefront of emerging scientific issues, and guidance on chemicals assessment will evolve to cover new ways of assessing hazards. The guidance provided in Chapter 4 is mainstream and reflects general consensus on how to assess existing industrial chemicals. However, additional guidance for specific groups of chemicals (e.g. metals), guidance on targeted chemical categories, investigation of adverse outcome pathways are areas under discussion; readers of Chapter 4 are also encouraged to consider publications in the OECD Series on Testing and Assessment where workshop reports and case studies illustrating emerging issues are regularly published.

[1] This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Existing Chemicals Programme up to April 2011.

4.2. GUIDANCE FOR THE INITIAL ASSESSMENT OF AQUATIC EFFECTS

4.2.1 Introduction

This section provides guidance for the initial assessment of aquatic effects of chemicals with data on some or all of the SIDS endpoints. It is based on the *Guidance Document for Aquatic Effects Assessment* (OECD 1995), which was developed reflecting the results of three OECD Workshops (OECD 1992a, 1992b and 1992c). These documents may be referenced whenever detailed information relating to the assessment procedure presented in this document is required. In particular, examples of effects assessments in OECD (1995) are useful for understanding the procedure and better reporting.

Useful guidance on the interpretation and assessment of aquatic hazardscan also be found in the third edition of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS):

Extensive guidance is also available from OECD countries. To cite a few examples, the European Chemicals Agency (ECHA) has developed guidance for registrants under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as well as for authorities evaluating the submissions (http://guidance.echa.europa.eu/). For example, guidance is available on information requirements for and interpretation of aquatic and sediment toxicity endpoints, related physical-chemical and environmental fate properties, as well as many other considerations related to assessing aquatic toxicity data. Another source of guidance is available from the U.S. Environmental Protection Agency under the Sustainable Futures Program can help to interpret ecotoxicity studies (http://www.epa.gov/opptintr/sf/). A useful document at the above website (under Training) is called *Interpretive Assistance Document for Assessment of Discrete Organic Chemicals* (http://www.epa.gov/opptintr/sf/pubs/iad_discretes_092009.pdf). There are also documents available from the U.S. Environmentla Protection Agency's Risk Assessment Forum that can be consulted – (http://www.epa.gov/raf/pubecological.htm).

NOTE: This section exclusively deals with the assessment of data already gathered. For testing requirements when elaborating a full (or partial) SIDS data set, Chapter 2 of this manual should be consulted. Once testing requirements are fulfilled or data gathered (in the case of a targeted assessment), all relevant SIDS and non-SIDS aquatic effects data on a chemical or category should be assessed.

This section focuses primarily on the initial aquatic effects assessment when a full set of SIDS data is available, but also references targeted assessments in some cases.

Full SIDS Data Set Assessments

In an initial assessment of all SIDS endpoints, the impact of the chemical is generally assessed against only one or two representative species from each of three trophic levels by means of short-term toxicity tests; i.e. using primary producers (algae), primary consumers (*Daphnia*) and secondary consumers (fish). A more refined assessment uses chronic or sub-chronic test data, as well as data on a larger number of aquatic species or data on terrestrial organisms. At the next stage of comprehensive effects assessment, (semi-) field studies provide the basis for assessments.

Therefore, the hazard endpoints that are relevant to this guidance are the following:

- acute toxicity to fish (normally a 96-hour test);
- acute toxicity to *Daphnia* (normally a 48-hour test); and

- toxicity to algae (normally a 72-hour test).

When the octanol-water partition coefficient (Log K_{ow}) is high, data on chronic toxicity to fish or daphnia may be available and should be assessed to determine the hazard to the aquatic environment.

Other ecotoxicity information such as toxicity to microorganisms, earthworms, terrestrial plants, birds and benthic organisms is also relevant in environmental effects assessment, but this guidance does not address such information in detail.

The following physical-chemical or environmental fate endpoints are used in initial aquatic effects assessment:

- partition coefficient (log K_{OW}), a SIDS endpoint;
- ready biodegradation (SIDS endpoint); and
- bioaccumulation (non SIDS endpoint).

In aquatic effects assessments used for risk assessment, the "low risk" concentration where no unacceptable adverse effects on the ecosystem are expected (i.e. Predicted No Effect Concentration, PNEC[2]) is often calculated, and it is compared with the concentrations that are present in the environment, either measured or calculated (i.e. Predicted Environmental Concentration, PEC). When the PEC exceeds the PNEC, further assessment or risk management action can be considered. As the aquatic effects assessment proceeds from the initial stage to refined and comprehensive stages (i.e. with data on multiple species and chronic toxicity data), estimation on PNEC becomes more precise with more detailed information made available. However in this programme PNECs are not generally derived from the available data, as approaches to their derivation can differ from country to country. Guidance for deriving a PNEC can be found in Annex 1 of this chapter. A full OECD SIDS assessment aims to present all available information to make derivation of PNEC possible, but will rarely present one. In addition, quantitative exposure information (i.e. estimation of PEC) is not a part of the usual process. However, some member countries may wish to conduct quantitative assessments as post-SIDS work.

This guidance is directly applicable to soluble compounds. Regarding poorly soluble compounds and other chemicals difficult to test, OECD (2000) provides guidance for testing the aquatic toxicity.

Targeted Assessments

Many of the same concepts related to the full SIDS endpoint assessments apply to assessments targeted on a limited number of SIDS or non-SIDS endpoints. For example, it is often necessary to consider physical-chemical and environmental fate endpoints in an assessment. However, the assessment might be limited to only one aquatic effects endpoint (e.g., bioaccumulation potential or acute fish toxicity).

4.2.2 Evaluation of data used for the assessment

Before conducting an effects assessment, data should be evaluated for their adequacy (see Chapter 3). Specific considerations for data evaluation described in OECD (1995) are summarised below.

[2] In OECD (1995), "maximum tolerable concentration"(MTC) is used instead of PNEC.

Octanol-water Partition Coefficient

The octanol-water partition coefficient (K_{OW}) is an important parameter in initial hazard assessment, and therefore should be examined carefully. For example, determination of K_{OW} by the shake flask method is not suitable for highly hydrophobic chemicals (log K_{OW} > 4). For those chemicals, the slow stirring method or generator column method can be used. A pH metric method was developed for ionisable substances (no OECD Test Guideline). Calculated Log K_{ow} values are also acceptable for most chemicals. It should also be noted that log K_{OW} is not applicable for surfactants, polymers, inorganics, organometalics and nanomaterials.

Biodegradation

Ready biodegradation is a SIDS endpoint relevant for organic chemicals. The information may give an indication of the potential persistence of the chemical. Although data from ready biodegradation tests are not used directly for the assessment of aquatic toxicity of a chemical, it is relevant to estimate the potential for aquatic organisms to be exposed in the long-term (i.e. if the chemical is not readily biodegradable). Both these aspects count in evaluating the overall hazard for the aquatic environment.

A series of test methods to determine the ready biodegradability of a chemical is available in OECD Test Guideline 301. Certain methods should not be used for insoluble or volatile chemicals unless precautions are taken. Generally the pass level required for a chemical to be considered readily biodegradable is 70% removal of dissolved organic carbon or 60% theoretical oxygen demand or carbon dioxide production. This pass level should normally be achieved within a 10-day window (the 10-day period after 10% biodegradation has occurred). Other biodegradation tests exist but they do not conclude on ready biodegradation.

Bioaccumulation

Bioaccumulation is not a SIDS endpoint but it is important for the prediction of the potential for chronic effects (and in the context of PBT (persistence, bioaccumulation and toxicity) assessment). Therefore bioaccumulation data should be reported when available; when not, a prediction based on physical-chemicals properties should be made.
Bioaccumulation occurs through multiple routes of exposure including uptake of food and sediment/soil, but for most organic substances with an appreciable solubility uptake from water (bioconcentration) is believed to be the predominant route of exposure. Data on bioconcentration (bioconcentration factor, BCF) can be obtained through the use of QSAR (e.g.,using K_{OW}) as well as by experiment. The OECD Test Guideline 305[3] describes an experimental protocol to determine bioaccumulation of a chemical. Care is needed in interpreting experimental results and special attention should be exercised on the validity of the test (fish growth during a study can influence results, lipid content of the fish should be relatively constant and may be influenced by feeding rate, age and weight of animals, etc.). Simple bioconcentration QSARs often cannot predict the BCF of extremely hydrophobic chemicals under field conditions. If more than one BCF is available for the same species, the geometric mean for the species could be used; however, the test concentration should be taken into account as results may be affected by any concentration dependence. BCF values are more often available for fish, but results may also be available for other species (blue mussel, oyster, scallop). Reported BCFs for microalgae should be used with caution. Guidance on the interpretation of bioaccumulation data can be found in OECD (2001a). The OECD follows the guidance provided in the Globally Harmonised System for Classification, Labelling and Packaging, where a BCF value of 500 is used to determine the potential for chronic effects in the absence of chronic data. Additional guidance is available for interpretation of BCF values (ECHA, 2008, Parkerton *et al*, 2008).

Where additional data are available (e.g., bioaccumulation factors, biomagnifications factor (BMF), biota-sediment accumulation factor (BSAF),, or trophic magnification factor (TMFs)), they can be used as a weight-of-evidence for an overall determination of bioaccumulation. There are several resources for describing these additional methods for determining bioaccumulation. (ECHA, 2008).

[3] The guideline is under revision and should in future include a method for determining bioaccumulation from dietary uptake of very hydrophobic chemicals in addition to bioconcentration.

Aquatic Toxicity

The water solubility of the test substance should be measured or predicted (if , 1 ppb) and it should be confirmed that effect concentration derived from the test does not significantly exceed the solubility limit. Test results using solvents (exceeding 100 µl/L) or dispersants should be treated with care. For further guidance of difficult substances, see OECD (2000).

For the interpretation of the data, the key aspects of the study methods that affect study quality, such as measured or nominal concentration, control response, use of sensitive vs. insensitive species, and water quality values, should be examined. Endpoints which have direct ecological relevance (e.g. survival, growth, reproduction) should be given more weight than other endpoints . Consideration of test species is also important; for example, chronic studies performed with the most sensitive species in the acute tests have highest relevance compared to chronic results with other species.

Chronic toxicity tests are much more relevant for persistent or bioaccumulative chemicals (i.e. when log K_{ow} is high). For some of these chemicals that are hydrophobic, acute exposure may not be sufficiently long to elicit effects. Also, for chemicals with a low water solubility, acute effects may not be observed at the limit of water solubility, while chronic effects would if the chemical is bioaccumulative. For these reasons, a chronic test on fish or aquatic invertebrate is strongly recommended for these substances.

If multiple data (acute or chronic toxicity data) are available for the same species, the following procedure is proposed for using the data:

- If these data are based on the same effect parameter (endpoint) and the same time period, the geometric mean value of results from the multiple studies should be used. The geometric mean is defined as GMy = $(y_1*y_2*y_3* \ ... \ *y_n)^{1/n}$, where y_{1-n} might be, for example, the NOECs from several 21-day *Daphnia* tests. The geometric mean minimises, compared to the arithmetic mean, the influence of highly deviating values.
- If different effect parameters or different exposure times are used, only the lowest value from the longest test time from a reliable test should be used taking into account the importance of the endpoints and the exposure periods in the various tests.

In the absence of chronic toxicity data, the bioaccumulation potential of a chemical may be used in combination with acute aquatic toxicity to evaluate the potential for chronic toxicity in aquatic organisms. Criteria used for hazard classification in the GHS are used to assess potential for chronic aquatic toxicity.

There is growing interest and data available for chemicals with endocrine active properties. A number of OECD Test Guidelines for aquatic toxicity testing are available for chemicals suspected to have endocrine active properties (OECD TG 229, 230, 231). When gathering data for the SIDS Dossier, available data on endocrine activity of the sponsored chemical should be reported in robust study summaries and results discussed in the assessment report. If results allow concluding on a hazard, the conclusion should be reported in the SIAP or ITAP.

QSAR Approach

In some cases, a SIDS element regarding aquatic toxicity or fate can be filled with (Q)SAR estimations. For further guidance on the use of (Q)SARs, see also Chapter 3. The most common estimation programmes used in the OECD Cooperative Chemicals Assessment Programme are EPISUITE (for e.g. environmental fate predictions), ECOSAR (for aquatic toxicity predictions), developed, owned and regularly updated by the United States Environmental Protection Agency. The OECD (Q)SAR Toolbox may also be used for a number of SIDS endpoints including, but not limited to, estimating aquatic toxicity. Adequate documentation of the estimation tools used should be available and reported in the SIDS Dossier, and in particular information on the applicability domain of the model used for the prediction and whether the sponsored chemicals is within the applicability domain. The assessment report should discuss the pertinence of using predictions (e.g., in the absence or in the impossibility of producing reliable test data).

QSARs can also be used to support test data or to decide which further data might be necessary (e.g., where test data might be missing for one trophic level which is predicted to be the most sensitive by (Q)SAR approach).

When QSARs are used, the approach and the reliability of the prediction should clearly be described in the equivalent of a study summary (e.g., QSAR Predictions Reporting Format (QPRF) and QSAR Model Reporting Format (QMRF)).

(Semi-) Field Test

Results from (semi-) field studies, (including short-term multi-species trials and long-term mesocosm trials), will not be available for many chemicals assessed in the OECD Programme on the Cooperative Assessment of Chemicals. Where they are available and are considered appropriate, they provide the basis for a comprehensive effects assessment in combination with chronic toxicity data.

Consideration of Indirect Effects Assessment and Assessment on Benthic Organisms

In addition to the effects assessments using pelagic aquatic organisms, assessments of indirect effects on birds and mammals through the ingestion of aquatic organisms and effects on benthic organisms (OECD 1992c) could be done if information on the chemical suggests possible hazard. Although such an assessment is usually beyond the data available for most chemicals assessed, there may be reasons that member countries may wish to evaluate indirect effects; for example, if a chemical has wide dispersive uses, the potential to bioaccumulate and mammalian repeated dose effects data that indicate a hazard. It is very rare to have chemicals with avian toxicity data, except for pesticides (and only these will have one or two test(s)).

Some methods mentioned in OECD (1995), USEPA (1984) and European Commission (2008), namely an approach using BCF for indirect effects and the equilibrium partitioning method for benthic organisms, could be considered. Fugacity modelling, indicating potential of a chemical to bind to e.g. soil or sediment together with a water-soil or water-sediment partitioning coefficient (K_{OC} or K_D) both give an indication of potential exposure of the benthic organisms to the sponsored chemical, and combined with aquatic toxicity studies, may give an indication of potential hazard to benthic organisms..

4.2.3 Reporting and interpretation of key results and assessment approaches

In SIARs as well as ITARs, the key study results and the assessment approaches and conclusions on potential hazard should be clearly stated.

Any deviation from standard test guidelines in the studies reported and used for the assessment should be discussed in the assessment report, as well as their impact on the results and any caution in interpreting results and assessing the hazard for the aquatic environment. For poorly soluble chemicals, attention should be paid when toxicity is observed at or slightly above the water solubility; the potential for bioaccumulation should be considered in determining the possibility of chronic effects in the absence of acute toxicity. If the conclusions of the initial assessment of a chemical suggest a concern with regard to aquatic effects (e.g., acute toxicity below 100mg/L and absence of ready biodegradation or bioaccumulation potential, or chronic toxicity below 1 mg/L), the conclusion should be clearly formulated in that sense. Standard language is proposed in Chapter 6 for conclusions in the SIAP and ITAP.

Although not mandatory in the SIDS assessment, a more precise assessment by elaborating exposure assessment, or by further testing, could be considered and proposed when a hazard to the aquatic environment is identified. For example, in cases where an estimated PNEC was derived applying assessment factors to the results of acute toxicity tests, performing chronic tests with appropriate species (e.g. most sensitive species in acute tests) would be considered as one of the possible further activities for member countries to consider. Also if there is a possibility of indirect effects on birds and mammals or a possible hazard to benthic organisms (as described above), assessments on these could be considered and proposed. The environmental partitioning of a chemical may also guide the need for further testing and assessment in other environmental compartments (e.g. soil or sediments). Although there is no agreed OECD guidance for the assessment of terrestrial effects, there are some guidelines for testing in soil and sediment organisms.

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4.3 GUIDANCE FOR THE INITIAL ASSESSMENT OF HEALTH EFFECTS[4]

4.3.1 Introduction

This document provides guidance for the initial assessment of health effects of chemicals with a full SIDS although the information and can be used to support targeted assessments. This document was first drafted based on relevant sections of the monographs of the International Programme on Chemical Safety (IPCS) (see list of references). These monographs can be consulted for information about making fuller assessments of chemical substances. Useful guidance on the interpretation and assessment of human health hazards can also be found in GHS documentation (United Nations, 2009).

Extensive guidance is also available from OECD countries and regions. One example is the guidance provided by the European Chemicals Agency (ECHA) for registrants under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as well as for authorities evaluating the submissions (http://guidance.echa.europa.eu/). Information can be found on general and specific information requirements related to human health endpoints, as well as many other considerations related to assessing human health data. Another example of guidance is available from the United States Environmental Protection Agency (US EPA): Risk Assessment Forum Documents from U.S. Environmental Protection Agency – http://www.epa.gov/raf/pubhumanhealth.htm .

When assessing the initial hazard of a substance with a full SIDS, evaluation should be focused on hazard identification, including the description and magnitude of adverse effects (dose (concentration) – response (effect) assessment:

- *Hazard Identification* aims to identify the effects of concern.
- *Dose (concentration) – Response (effect) Assessment* is the estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect. At this step the no observed adverse effect level/concentration (NOAEL/NOAEC), or if this is not possible, the lowest observed adverse effect level/concentration (LOAEL/LOAEC), shall, where possible and appropriate, be determined for the observed effects. If appropriate, the shape of the dose-response curve should also be considered.

It should be noted that this section deals with the assessment of all relevant available information. For testing and data requirements when elaborating a full SIDS (or for specific endpoints within targeted assessments), chapter 2 of this manual should be consulted.

For many chemicals currently being assessed at OECD, there will be data available in excess of SIDS; these additional data should, of course, be assessed and taken into consideration when developing conclusions and recommendations. Also, for targeted assessments, endpoints not commonly assessed (e.g., carcinogenicity, neurotoxicity) might be the focus of the assessment. However, in making the initial assessment of health effects for chemicals with a full SIDS, the elements in the SIDS that are relevant in this respect are:

- Acute Toxicity;

[4] This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Existing Chemicals Programme up to April 2011

- Repeated Dose Toxicity;
- Genetic Toxicity;
- Reproductive toxicity, and
- Developmental Toxicity.

Furthermore, this document also gives guidance on the following non-SIDS endpoints, when test results are available:

- Toxicokinetic (see Annex)
- Irritation to Skin and Eyes
- Sensitisation
- Carcinogenicity
- Neurotoxicity

In the full assessment of repeated dose toxicity and reproduction/developmental toxicity, Uncertainty Factors (UFs) are used and the Estimated Level of Low Concern (EDLC) – also called Reference Dose (RfD) or Acceptable Daily Intake (ADI) – is calculated from the No-Observed-Adverse-Effect level (NOAEL) or, when not available, the Lowest Observed-Adverse-Effect level (LOAEL) derived from animal test results. In some domestic programmes, the benchmark dose (BMD) approach is even used in lieu or in addition to the NOAEL/LOAEL or NOAEC/LOAEC as the BMD modelling takes into account the shape of the dose-response curve, not only the dose tested. However, in the context of the OECD Programme on the Cooperative Assessment of Chemicals, the derivation of EDLCs and hence the use of UFs are usually not made as the programme is limited to initial assessment.

4.3.2 Acute Toxicity

In the assessment of the toxicity of a chemical, the determination of the acute toxicity is often the first step. Generally the objectives of investigating the acute toxicity are to determine the following (ECHA, 2008):

- Whether a single (or multiple exposures within 24 hours) to the substance of interest produce lethality or could be associated with adverse effects on human health;; and/or
- the types of toxic effects that are induced following a single exposure to a substance by the oral, dermal or inhalation route, their time of onset, duration and severity (all to be related to dose/concentration); and/or
- the dose/concentration-response relationship and, when available, the slope of the dose-response curve; and/or
- when available, any marked sex differences in response; and
- any information necessary for classification and labelling of the substance for acute toxicity.

In the context of the OECD Programme on Cooperative Assessment of Chemicals, data on acute toxicity will usually not lead to recommending action for follow-up testing, although exceptional findings (high lethality, neurotoxicity seen at low doses, etc.) may warrant such action.

A variety of OECD guidelines for testing chemicals (as well as guidelines from other organisations and governments) have been developed for measuring acute toxicity. OECD guidelines are publicly available (http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html). Several guidance documents that may assist in evaluating acute toxicity are also available from OECD. These include biostatistical performance of the toxic class (OECD TG 436) method for acute inhalation toxicity, use of acute toxicity tests, comparison of concentration by time and the 403 protocol as well as considerations for estimating acute reference doses (2001b; 2001c; 2009a, 2009b, 2009c; 2010a).

4.3.3 Irritation

No testing for skin or eye or respiratory tract irritation is required under the OECD Programme on Cooperative Assessment of Chemicals. Available test results should nevertheless be described and assessed. These hazard endpoints are very relevant for the workplace and consumers, and additional guidance is available elsewhere (e.g. ECHA (2008)) if needed. The general objectives are to determine:

- whether the substance is, or is likely to be, corrosive;
- whether animal or *in vitro* studies indicate evidence of significant skin, eye or respiratory irritation;
- whether there are indications of skin, eye mucous membrane or respiratory irritation following human exposure to the substance;
- the time of onset, extent and severity of the responses and reversibility.

If results from *in vivo* animal studies are available, for adequate hazard identification, information on the local responses (erythema and/or oedema for skin; corneal opacity, iridal effects, conjunctival redness and/or swelling for the eye) following application of a single defined amount of the substance should be reported. However, there is no OECD Test Guideline for the respiratory tract irritation. The local responses are evaluated and graded for each exposed animal at specified intervals after application of the test substance. Information should also be reported on the time fully to establish reversibility (or on the lack of reversibility), on any other local effects (e.g. pain, ocular discharge, necrosis, irreversible coloration of eyes) or any other toxic effects. For respiratory tract irritation, information from acute and repeated dose inhalation toxicity studies will often be considered. Also, it is usually assumed that corrosive (and severely irritating) substances would also cause respiratory irritation when vaporised or in form of aerosol. Furthermore, information from human cases where symptoms have been described associated with occupational exposures can be used on a case-by-case basis to characterise the respiratory irritation potency of a substance.

Appropriate details for interpretation can be found in several OECD Test Guidelines. OECD TG 404 and 405 refer to the classic *in vivo* guidelines for skin and eye irritation, respectively. Additional guidelines are available for *in vitro* tests. For skin irritation, these include OECD TGs 430, 431, 435 and 439. Draft revised guidelines are also available for OECD TGs 430 and 431. For eye irritation, *in vitro* tests are described in OECD TGs 437 and 438. These guidelines can be found at http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html.

In addition, OECD guidance documents may also be consulted to assist in the interpretation of irritation data. OECD (2009d) discusses the criteria for validation and acceptance of alternate test methods, with emphasis on testing strategies for skin and eye irritation. The scientific basis, validation data, and applicability under UN GHS and new GHS-compliant performance standards of reconstructed human epidermis methods are addressed in OECD (2010b).

4.3.4 Sensitisation

A sensitiser is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin or by inhalation the characteristic adverse health effects of allergic contact dermatitis or asthma (and related respiratory symptoms such as rhinitis), respectively, may be provoked.

No testing for sensitisation is required under the OECD Programme on the Cooperative Assessment of Chemicals. Available test results should nevertheless be described and assessed.

When evaluating human data, attention should be paid to:

- the number of well-documented cases in relation to the size of the exposed population;

- the relevance of any described cases and the association between clinical symptoms and clinical test results and exposure;
- the type of exposure (including: adequate substance identification, frequency, duration and magnitude of exposure, the physical state of the substance and exposure to other structurally-related substances). Data from subjects where exposure was not to intact skin or from subjects with pre-existing asthma should be interpreted with caution;
- the quality of the epidemiological data.

The use of human volunteers in chemical risk assessment is a controversial issue, with a range of views and regulatory requirements held by different OECD member countries. Therefore individual countries will select appropriate values and data dependent on their specific regulatory requirements or risk management policies.

Particular points to take into account when evaluating results from animal assays to predict skin sensitisation include:

- choice of vehicle;
- skin irritation at the induction phase of guinea pig tests;
- maximal non-irritating concentration at the challenge phase of guinea pig tests;
- signs of systemic toxicity;
- dose response and statistical analysis in case a local lymph node assay (LLNA) was performed;
- effects observed in both a positive (with a known sensitizer) and a negative (vehicle) control group.

Assessment of cutaneous reactions at the challenge phase of guinea pig tests should be conducted carefully to discriminate irritation from sensitisation. Further guidance can be found in ECHA (2008).

Available OECD guidelines include OECD TG 406 (guinea pig maximization and Buehler test methods) and 429, 442A and 442B (local lymph node assay using radioactive and non-radioactive methods) (http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html). These guidelines can be consulted for proper methods and criteria with which to assess available studies. In addition, information on the theory and acceptability of the methods is briefly presented in the guidelines.

4.3.5 Repeated Dose Toxicity

The primary objective of assessing repeated dose toxicity is to identify and describe both general (e.g. body weight changes) and specific (target organ) adverse effects and their severity, including dose/concentration-response characteristics that may be associated with the chemical being reviewed. Generally, animal (rodent) studies are at least 28-days in duration and the animals are exposed to a number of dose/concentrations of the test chemical plus one or more control(s). These doses/concentrations are then used to derive a value for the No-Observed-Adverse-Effect level/concentration (NOAEL/NOAEC), or the Lowest Observed-Adverse-Effect level/concentration (LOAEL/LOAEC). The NOAEL/NOAEC is considered to be the highest daily dose or concentration of a substance at which there is no adverse alteration observed in the morphology, functional capacity, growth, development, etc. of the target. The LOAEL/LOAEC, on the other hand, is considered to be the lowest daily dose or concentration of a substance at which any of these adverse alterations is actually observed. In general, greater confidence for assessing the hazards of a substance is placed in a NOAEL/NOAEC than in a LOAEL/LOAEC; in a NOAEL/NOAEC obtained from a sub-chronic study rather than one from a sub-acute study; in a test which demonstrates a clear dose-response relationship; and in a test in which the manifestations of toxicity are well-defined. In principle, a NOAEL/NOAEC should be obtained in each repeated dose study and can be used to derive a standard considered to represent a level

of exposure or dose at which it is believed there is little if any likelihood of adverse effects in humans. However, when a reliable dose-response relationship is obtained, and a NOAEL/NOAEC cannot be estimated, a LOAEL/LOAEC could be used if the fact that the LOAEL/LOAEC is being used is clearly stated and consideration is being given to the slope of the dose-response curve.

As an alternative to this "classical" NOAEL/NOAEC approach, where feasible the so-called "bench-mark dose" approach could also be adopted. However, as this latter system uses the lower confidence limit of the dose corresponding to the lowest increase judged to be toxicologically significant in the incidence of an effect, it is anticipated that the number of repeated dose studies where adequate quantal or continuous information is available will be limited. For more guidance on the "bench-mark dose", see US-EPA (1995), Slob & Pieters (1998) and EPA (2000).

Crucial in the dose-response assessment, is the definition of "adverse effects". In repeated dose toxicity testing, the values of selected parameters are compared to the average values in untreated concurrent control animals. Adverse effects cannot be defined in purely statistical terms as significant changes relative to control values. A judgement regarding biological significance is necessary. What is considered to be an adverse effect is dependent on expert judgement. In those cases where an adverse effect is observed in, for example, a parameter which monitors an organ system, such as a clinical biochemical change in a measurement of liver function, more weight can be attributed to its significance if other observations for that organ system, such as necropsy and histopathology findings and to a lesser extent organ weight difference, also indicate an adverse effect. In addition, the dose response of an adverse effect, i.e. the progression of a change in an organ system with the dose, is a factor which adds weight to the significance of the effect. It should be kept in mind that some of the tests approved for fulfilling SIDS elements [e.g. according to TG 421, 422] are screening tests. It may therefore be that only one dose, either the limit dose or the highest testes dose provides data that suggests an adverse effect. Under such circumstances careful professional judgement is required to determine if such an effect is probable in the absence of dose-response or even statistical significance. In study designs where the data are sufficiently robust, other aspects to be considered include reversibility of the toxicity, severity of the effect, latency of the onset of the effect and the shape of the dose-response curve. Correlations observed between changes in several parameters, e.g. between clinical or biochemical and (histo)pathological effects, will be helpful in the evaluation of the adversity of effects.

The decision as to whether or not a local effect should be considered as a substance-related adverse effect or caused by treatment procedures (e.g. adverse effects in the upper gastro-intestinal tract, mediastinum and lungs following bolus application in oral gavage studies), should be based on expert judgement. If local effects are clearly identified after repeated dosing, a NOAEL/NOAEC or LOAEL/LOAEC should be established for these effects in addition to N(L)OAELs/N(L)OAECs for systemic effects.

More guidance on the analysis and evaluation of repeat-dose toxicity studies can be found in OECD (2000).

4.3.6 Genetic Toxicity

Testing for genetic toxicity is conducted so that chemicals may be assessed for their potential to cause transmissible damage to the genetic material of somatic cells (with potential carcinogenic or other consequences) and germ cells (which may result in heritable damage to the offspring).

It is essential to differentiate between the *in vitro* tests, which are primarily used to investigate intrinsic potential of chemicals to cause genetic damage and the *in vivo* tests, which investigate if these intrinsic properties are expressed in whole animals.

For the initial assessment, results of at least two tests for genetic toxicity will generally be provided in the SIDS. These are expected to include results of an *in vitro* point mutation test and a test for structural

chromosomal damage (either *in vitro* or *in vivo*). A wealth of other genotoxicity data may be available from studies conducted *in vitro* and/or *in vivo*, but many of these tests may have been conducted using methods different from the standard OECD Test Guidelines. The validity and usefulness of each of the data sets to the overall assessment of genotoxicity should be individually assessed, taking account of protocol design (including route of administration) and current expert views on the value of the test systems.

Evaluation of genotoxicity test data should be made with care, taking into account all available information. Particular points to take into account when evaluating "negative" test results include:

- the doses or concentrations of test substance used (were they high enough?);
- the volatility of the test substance (were concentrations maintained in tests conducted *in vitro*?);
- for *in vitro* studies, the possibility of metabolic activation or deactivation was not assessed in the system;
- the bioavailability of the substance to the target organ;
- the reactivity of the substance (e.g. rate of hydrolysis, electrophilicity, presence or absence of structural alerts and other available indications related to potential mutagenic activity of the chemical structure);
- the response of the positive and negative controls (important to both *in vitro* and *in vivo* assays).

Contradictory results between different test systems should be evaluated with respect to their individual significance. Examples of points to be considered are as follows.

- Conflicting results obtained in non-mammalian systems and in mammalian cell tests may be addressed by considering possible differences in metabolism or in the organisation of genetic material. Additional information may be needed to resolve contradictions.
- Positive results in the *in vitro* SCE assay should be viewed with caution, as this assay is associated with a relatively high incidence of false positive results. In addition, the SCE-formation is not clearly understood. Also since SCE are an indication of effects on DNA and not necessarily on chromosomes, a positive result in this assay would not be considered to be evidence of a significant clastogenic potential *in vitro*, especially if negative results were available in an *in vitro* chromosome aberration assay.
- Similarly, interpretation of results from DNA binding assays should be viewed with caution as these assays are only considered to be indicators of DNA damage. Consequently, the observance of *in vitro* DNA adducts alone in the absence of positive findings from *in vivo* assays is generally not considered sufficient evidence of a significant genotoxic potential *in vivo*.
- If contradictory findings are obtained *in vitro* and *in vivo*, in general, the results of *in vivo* tests indicate a higher degree of reliability. However, for evaluation of "negative" results in *vivo*, it should be considered whether there is adequate evidence of target tissue exposure.

The consequences of "positive" findings only at highly toxic/cytotoxic concentrations, and the presence or absence of a dose-response relationship should be considered. The default assumption for genotoxic chemicals, in the absence of mechanistic evidence to the contrary, is that they have a linear dose-response relationship. However, both direct and indirect mechanisms of genotoxicity can be non-linear or threshold, and so sometimes the default assumption may be inappropriate. When interpreting positive results, considerations of the dose-response relationship and of possible mechanisms of action are important components of a hazard assessment. Examples of mechanisms of genotoxicity that may be demonstrated to lead to non-linear or threshold dose-response relationships include extremes of pH, ionic strength and osmolarity, inhibition of DNA synthesis, alterations in DNA repair, overloading of defence mechanisms (anti-oxidants or metal homeostasis), interaction with microtubule assembly leading to aneuploidy, topoisomerase inhibition, high cytotoxicity, metabolic overload and physiological perturbations (e.g. induction of erythropoeisis).

In general and especially for the purpose of the OECD Cooperative Chemicals Assessment Programme, substances for which both the *in vitro* point mutation test and the *in vitro* chromosomal aberration test are negative can be considered as non-genotoxic.

Substances, for which positive test results are available, are usually considered to be of concern. Two actions are possible: 1) form a conclusion based only on the *in vitro* genotoxicity data of the chemical, or 2) conduct further testing *in vitro* and possibly also *in vivo* to further investigate the hazard detected *in vitro* and form a conclusion on genotoxicity based upon both *in vitro* and *in vivo* data.
However, if only *in vitro* test results are available and one of the two *in vitro* tests is positive, further work is usually necessary within the SIDS context:

- When the mammalian cell test *in vitro* is negative, it will be necessary to decide whether further work is needed on a case-by-case basis. Further testing could be either *in vitro* or *in vivo*. Suspicion that the positive response observed in the bacterial test was due to a specific bacterial metabolite of the test substance could be explored further by investigation *in vitro*. Alternatively, an *in vivo* test may be required.
- Following a positive result in an *in vitro* mammalian cell mutagenicity test, adequately conducted *in vivo* testing, such as the micronucleus test, the bone marrow chromosomal aberration test or a transgenic rodent assay is usually required to ascertain if this potential can be expressed *in vivo*. In exceptional cases, where it can be sufficiently deduced that a positive *in vitro* finding is not relevant for *in vivo* situations, *in vivo* testing may not be necessary.

Before undertaking any *in vivo* testing, a review of the *in vitro* test results and all available information on the toxicokinetic and toxicodynamic profile of the test substance is needed, as well as consideration of available information about structure-activity relationships. A particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol (using the most appropriate route of administration) that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. Further information on assessment of in vitro positive results in genetic toxicology tests is available in a review paper by ILSI/HESI (Dearfiled *et al*, 2011).

If necessary, an investigation of toxicokinetics could be conducted before progressing to *in vivo* testing. If the *in vivo* test is negative with limitations clearly identified on the interpretation of the test (e.g. test chemical not reaching the target organ), the need for further work could still be considered (such as testing in a second tissue to supplement a negative *in vivo* assay when positive results have been seen in an *in vitro* point mutation assay). In this regard, attention should be paid to the quality and relevance of all the available data, the adequacy of target tissue exposure and the potential for human exposure.

Further information introducing genotoxicity Test Guidelines is being revised and should soon be available in the OECD Series on Testing and Assessment or under Test Guidelines. However there is currently no agreed OECD guidance beyond what is provided here in this Manual on the assessment of genotoxicity. Additional information on genetic toxicity test batteries is available in Cimino (2006) and Eastmond *et al* (2009).

4.3.7 Reproduction/Developmental Toxicity

Reproduction toxicity represents any effect on fertility and reproduction that can adversely affect the continuation of the species. Developmental toxicity is any adverse effect induced during the developmental period, i.e. from conception through puberty. The major manifestations of developmental toxicity include death of the developing organism, structural abnormalities, altered growth and functional deficiencies.

Developmental toxicity can be considered a component of reproductive toxicity, and sometimes it is difficult to distinguish between effects mediated through the parents versus direct interaction with developmental processes.

The organisation of the information for an assessment of reproduction and developmental toxicity is described in a number of OECD Test Guidelines related to these endpoints (TG 414, 415, 416) and the guidelines for the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (TG 422), the Reproduction/Developmental Toxicity Screening Test (TG 421), and the Developmental Neurotoxicity Guideline (OECD 426). Toxic response data should be considered by sex and dose and, when possible, be sub-divided into reproductive and developmental effects. Reproduction effects would include, *inter alia*, altered fertility indices for males and females, effects on mating performance or other factors affecting reproductive function and, when related to the nursing capacity of the females, postnatal viability indices for the offspring or other postnatal signs of toxicity. Developmental effects, either as a consequence of maternal toxicity or as a direct effect on the developing organism, would include, *inter alia*, decreased numbers and percentages of live offspring per litter, and increased numbers and percentage of affected offspring (male, female or combined) per litter. Data on maternal toxicity and on certain metabolic or kinetic observations need to be considered when determining the nature, severity and relevance of developmental toxicity.

Reproductive and developmental effects typically exhibit dose-response relationships, and where these effects are not genotoxic (e.g. heritable) thresholds are generally assumed to exist. It is thus possible to estimate exposure levels unlikely to produce effects in humans on the basis of a NOAEL obtained in an animal experiment, in a similar manner to that for repeated dose toxicity.

The occurrence of a dose level producing well defined toxicity is considered of crucial importance in reproductive and developmental toxicity studies. This is called for in the OECD Test Guidelines for both screening tests, 421 and 422. Tests in which toxicity is not observed should, therefore, not be considered as adequate tests unless the limit concentration of 1000 mg/kg bw/d or a higher dose level (when relevant) has been included.

In addition, useful information can be derived from the repeated dose toxicity study, e.g. pathology in the reproductive organs, if specific histological examination has been carried out and a comparison of dose-response curves for such an effect between males and females could be made both in the repeated dose toxicity and the reproduction toxicity study.

To satisfy the SIDS requirements for reproductive toxicity, information (e.g. test data from studies in animals) is required which addresses both reproductive parameters (including fertility) and developmental toxicity. Examples of acceptable information are provided below:

- Requirements are met if existing data on the chemical include a developmental toxicity study and a 90-day (or longer) repeated dose study that sufficiently documents that reproductive organs were examined histologically and indicate no effects. If results from a developmental toxicity study are not available then such a study is required (e.g. OECD Test Guideline 414).

- When either a ≥ 90-day (with no evaluation of reproductive organs) or a 28-day repeated dose study is the only repeated dose study available, it is recommended that at least a reproduction/developmental toxicity screening test (e.g. OECD Test Guideline 421) be carried out, in order to satisfy the requirements for the reproductive/ developmental toxicity endpoint.

- When a repeated dose toxicity test of 28-days or longer is not available, then a combined repeated dose toxicity test with a reproductive/developmental screening test (e.g. OECD Test Guideline 422) can be carried out to satisfy the requirements for repeated dose and reproductive/developmental toxicity. (This option uses the lowest number of test animals to satisfy both the repeated dose and the reproduction toxicity requirements.)

- If reliable tests results from well performed tests according to OECD Test Guidelines 415 or 416 (one or two generation reproductive toxicity) are available, the SIDS requirements for reproductive/developmental toxicity are met.

Data from animal studies ideally should provide clear evidence of specific reproductive toxicity in the absence of other, systemic, toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects should be assessed to the fullest extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternal toxic doses should not be automatically discounted. Discounting developmental effects that are observed at maternal toxic doses can only be done on a case-by-case basis.

Overall Evaluation of Maternal and Developmental Toxicity

Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern because the developing organism is affected but toxicity is not apparent in the adult. However, the more common situation is when adverse developmental effects are produced only at doses that cause minimal maternal toxicity; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. At doses causing excessive maternal toxicity (that is, significantly greater than the minimal toxic dose), information on developmental effects may be difficult to interpret and of limited value. Current information is inadequate to assume that developmental effects at maternally toxic doses result only from maternal toxicity; rather, when the LOAEL is the same for the adult and developing organisms, it may simply indicate that both are sensitive to that dose level. Moreover, whether developmental effects are secondary to maternal toxicity or not, the maternal effects may be reversible while effects on the offspring may be permanent. These are important considerations for agents to which humans may be exposed at minimally toxic levels either voluntarily or involuntarily, since several agents are known to produce adverse developmental effects at minimally toxic doses in adult humans (e.g., smoking, alcohol, isotretinoin).
Since the final assessment not only takes into account the potential hazard of an agent, but also the nature of the dose-response relationship, it is important that the relationship of maternal and developmental toxicity be evaluated and described, if possible. Then, information from the exposure assessment is used to determine the likelihood of exposure to levels near the maternally toxic dose for each agent and the risk for developmental toxicity in humans. Although the evaluation of developmental toxicity is the primary objective of standard studies within this area, maternal effects seen within the context of developmental toxicity studies should be evaluated as part of the overall toxicity profile for a given chemical. Maternal toxicity may be seen in the absence of or at dose levels lower than those producing developmental toxicity. If the maternal effect level is lower than that in other evaluations of adult toxicity, this implies that the pregnant female is likely to be more sensitive than the nonpregnant female. Data from reproductive and developmental toxicity studies on the pregnant female should be used in the overall assessment of risk.

Guidance on the histologic evaluation of endocrine and reproductive tests in rodents is available in OECD (2009e). Additional guidance on mammalian reproductive toxicity testing and assessment is available in OECD (2008). There are also some general guidelines from the U.S. Environmental Protection Agency's

Risk Assessment Forum that can be used as references. Although the EPA guidelines have been available for some time (1996 and 1991, respectively), the essential elements and scientific opinions expressed are still applicable and very useful when interpreting reproductive and developmental toxicity data. These documents are available at the following links: http://www.epa.gov/raf/publications/pdfs/REPRO51.PDF and http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF. .

4.3.8 Carcinogenicity

Detailed guidance is provided in OECD (2010c), EPA (2005a) and EPA (2005b) on the assessment of carcinogenicity studies. Carcinogenicity is not a SIDS element, and information is rarely available for substances assessed under the OECD Cooperative Chemicals Assessment Programme, unless there are reasons to suspect the substance is a known or a potential carcinogen, in which case, studies should be available for review. If information on the potential carcinogenicity is available for a substance, it should be described and assessed in the SIDS Documents in the same way as information on a SIDS element.. An IPCS framework for analysing the relevance of cancer modes of action for humans has been published along with three case studies illustrating its application (WHO, 2008, and Boobis *et al*, 2006).These harmonised approaches should be taken into account when assessing existing tests results on carcinogenicity in the SIAR. If internationally agreed assessments are available (e.g. by IARC), the conclusions of those assessments should be reflected in the SIAR.

4.3.9 Neurotoxicity

No detailed guidance on the assessment of neurotoxicity is provided in this document. Neurotoxicity is not a SIDS element in itself, and detailed information is rarely available for substances assessed under the OECD Cooperative Chemicals Assessment Programme. If information on the potential neurotoxicity is available for a substance (e.g. through functional observations noted in a 90-day study or similar repeated dose toxicity studies), it should be described and assessed in the SIDS Documents in the same way as information on a SIDS element. Guidance on the assessment of neurotoxicity studies can be found in IPCS (2001a). Guidance is also available from the Risk Assessment Forum at the U.S. Environmental Protection Agency (US EPA) that can be consulted: http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF .

4.3.10. Endocrine Disruption

Endocrine-related endpoints are an area of concern in chemicals assessment and *in vitro* and *in vivo* test methods covering such endpoints are available (e.g., OECD TG 407, TG 440, TG 441, TG 455). Results of the studies listed above are meant to assist in understanding the mode or mechanism of action of results obtained in other studies such as reproductive or repeated-dose toxicity studies. A guidance document for the assessment of test results from endocrine-related endpoints is under preparation and should soon be published in the OECD Series on Testing and Assessment.

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ANNEX1

CALCULATION OF PNEC

This annex provides a description of how PNEC can be derived from a SIDS assessment. Two approaches are described: the assessment factor (deterministic) approach and the Species Sensitivity Distribution (probabilistic) approach.

Assessment Factors Approach

The assessment factor method is the method most usually used for the derivation of a PNEC when only acute toxicity data or limited chronic toxicity data are available. A PNEC is calculated using toxicity test data such as LC_{50}, EC_{50}, other $L(E)C_x$ values, NOEC (no observed effect concentration) and LOEC (low observed effect concentration). MATC (maximum allowable toxicant concentration, calculated as MATC = $(NOEC \times LOEC)^{1/2}$) is also used in effects assessment.

Assessment factors are used to adjust the effect concentration from a limited data set and to estimate a PNEC. Assessment factors should reflect the following uncertainties and extrapolations:

- intra-species and inter-species variations;
- inter-laboratory variations;
- the extrapolation of short term toxicity towards long term toxicity; and
- the extrapolation of laboratory results towards the field.

Assessment factors should be applied with care to acute data for substances which are suspected of having a specific mode of action.

Different assessment factors are used in different methodologies for a particular dataset. These are outlined below. In the following paragraphs, assessment factors that could be used in estimating PNEC from SIDS data are described. These are summarised in Table 1. When targeted assessments are presented that evaluate limited numbers of endpoints, PNECs may not often be determined. However, if there is a reason to provide protective measures for the endpoint in question, then several of the considerations within this section could apply to the assessment.

When only acute toxicity data in the SIDS are available, an assessment factor of between 100 and 1000 is applied to the lowest $L(E)C_{50}$ [i.e. case (a)]. A factor of 1000 is a conservative and protective factor and applied when only limited data are available, i.e. this value may be reduced to 100 if evidence is available to suggest that this may be a more appropriate factor. Such evidence would include:

(1) data from a wide variety of species including those which are considered to represent the most sensitive species;

(2) information, from structurally similar compounds or QSAR, to suggest that the acute to chronic ratio is likely to be low;

(3) information to suggest that the chemical acts in a non-specific or narcotic manner, with little inter-species variation in toxicity; and

When chronic toxicity data are available in addition to acute data, an assessment factor of between 10 and 100 is applied to the lowest NOEC [i.e. case (b)], taking the following situations into account:

(1) If chronic NOEC is available from one or two species representing one or two trophic levels (i.e. fish, *Daphnia* or algae) identified as the most sensitive in the acute toxicity studies, a factor of 100 or 50 is applied to the lowest NOEC. In this case, a PNEC value derived from chronic data should be compared to that derived from the lowest acute data. The lowest PNEC value is then used in the assessment.

(2) If chronic NOECs are available from three species representing three trophic levels (i.e. fish, *Daphnia* and algae), a factor of 10 is applied to the lowest NOEC. If there is convincing evidence that the most sensitive species has been tested, a factor of 10 may also be applied to the lowest NOEC from two species representing two trophic levels (i.e. fish and/or *Daphnia* and/or algae). On occasions, the assessment factor may be lower than 10 when the database is large, covers long-term effects (e.g. multi-generation tests), etc.

Use of different assessment factors should be clearly justified in the assessment report.

Table 1. Summary of Proposed Assessment Factors for Estimating an PNEC

Case	Data available	Range of Assessment factor
(a)	EC_{50} algae (72hr) EC_{50} *Daphnia* (24-48hr acute test) LC_{50} fish (96hr)	100 - 1000
(b)	NOEC *Daphnia* (14-21d chronic toxicity test) NOEC algae (72hr) NOEC fish (chronic toxicity test)	10 - 100

Assessment factors proposed in literature

Several sets of assessment factors have been proposed to date. At an OECD workshop, (OECD 1992b), a factor of 10 is suggested for each extrapolation step described in paragraph 16 in Section 4.2.3 of this manual. This approach is a modification of a method proposed in USEPA (1984).

Assessment factors proposed in REACH guidance (2008) depend on the properties of the chemical (e.g. if log K_{OW}>5 an additional assessment factor of 10 is applied for soil and sediment PNECs; this accounts for the potential for exposure to the substance adsorbed to particles as well as dissolved in the pore water). In Heger et al. (1995), a factor of 100 between the $E(L)C_{50}$ of acute toxicity and NOEC of chronic toxicity has been shown by measured data to be generally justifiable.

The proposals from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 1993) are based on comparisons of empirical toxicity data for x chemicals??. An acute: chronic ratio of 40, a chronic: ecosystem ratio of 5, and an ecosystem : field ratio of 1 are suggested.

Table 2 summarises these proposals. These factors can be modified under certain conditions (e.g. an assessment factor of 1000 in the EU Technical Guidance Document can be lowered to 100 with certain evidence). The original reference should be referred to for detailed explanation of such modifications.

Table 2. Proposed Assessment Factors for Application to Aquatic Toxicity Data for Estimating a PNEC

Available information applied	**Assessment factor applied to the lowest value (modifications not included)**		
	(a) OECD Workshop	(b) REACH guidance	(c) ECETOC proposal
One acute $L(E)C_{50}$ for acute toxicity from one trophic level	1000	-	-
At least one acute[5] $L(E)C_{50}$ from each of three trophic levels of the base-set (fish, *Daphnia* and algae)	100	1000	200
One chronic NOEC (either fish or *Daphnia*)	-	100	-
Two chronic NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	-	50	5
Chronic NOECs from at least three species (normally fish, *Daphnia* and algae) representing three trophic levels	10	10	
Field data or model ecosystems	-	case-by-case	1

Statistical Extrapolation Methods Approach

If a large data set from long-term tests for different taxonomic groups is available (OECD, 1992), statistical extrapolation methods may be used to derive a PNEC. The main underlying assumptions of the statistical extrapolation methods are as follows (OECD, 1992b):

- The distribution of species sensitivities (SSD) follows a theoretical distribution function;
- The group of species tested in the laboratory is a random sample of this distribution.

[5] In the EU Technical Guidance Document, "short-term toxicity" and "long-term toxicity" are used instead of "acute toxicity" and "chronic toxicity."

The effects assessment can be performed with a statistical extrapolation method if the database on species sensitivity distributions (SSDs) is sufficient for its application (Posthuma et al., 2001). This approach has been applied for Nickel and nickel compounds in the OECD HPV Chemicals Programme in 2007. The assessment conclusions are available in the Existing Chemicals database.

In general, long-term toxicity data are log-transformed and fitted according to the distribution function and a prescribed percentile of that distribution is used as a criterion. Several distribution functions have been proposed for environmental species. The EPA (1984) assumes a log-triangular function, Kooijman (1987) and Van Straalen and Denneman (1989) a log-logistic function, and Wagner and Løkke (1991) a log-normal function. Aldenberg and Slob (1993) refined the way to estimate the uncertainty of the 95th percentile by introducing confidence levels, which was again more refined by Aldenberg and Jaworska (2000). This is an area where further harmonisation of practice is needed at the level of OECD member countries.

An advantage of these methods is that they use the whole sensitivity distribution of species in an ecosystem to derive a PNEC instead of taking always the lowest long-term NOEC. However, the probabilistic method has been criticised for a lack of transparency compared to the deterministic approach, the question of the representativity of the selected test species, the comparability of endpoints, the arbitrary choice of a specific percentile and a statistical confidence level, etc. On the other hand, the SSD approach uses all available valid long-term data and so may provide a more realistic measure of effect threshold levels (assuming that the coverage of species is sufficiently great). In addition, an assessment factor is still applied to the derived SSD effect value, taking into account the quantity of data used in its derivation.

When using a statistical extrapolation method to derive a PNEC, the following issues need to be addressed:

- Clarification of the type of input data, i.e. preferably reliable NOECs from chronic/long-term studies, full life-cycle or multigeneration studies;
- Information on the mode of action of the substance that may help to identify and to evaluate the need to include possible sensitive taxonomic groups or to exclude possible overrepresentation of certain taxonomic groups;
- The minimum species requirements, e.g. representative species from the following taxonomic groups: fish, crustaceans, insects, algae, higher plants, other groups not already represented. It is recognised that for some taxa mentioned above, no internationally standardised test guidelines for long-term tests are currently available. The requirement can be adapted based on knowledge/reasoning about sensitive endpoints and species as well as knowledge on structure – activity and mode of action.
- The minimum sample size (number of data) to establish a species sensitivity distribution curve. Differing guidance is provided in different for a. OECD (1992b) proposes a minimum of 8 NOECs on species from different taxonomic groups, EC (2002) and ECHA (2008) recommend 10 NOECs (and preferably more than 15) on species from 8 taxonomic groups. Similar proposals have been made by Gibbons and Coleman (2001) or de Bruijn et al. (1999).
- The method for handling multiple data for one species, e.g. averaging comparable data, or selecting the most sensitive endpoint when various endpoints are observed;
- Statistical fitting procedures, i.e. the method must be mentioned and explained, where the log-normal distribution is the preferred one for pragmatic reasons. In addition, a statistical method is to be used to test the goodness of fit. In addition to the Kolmogorov-Smirnov test, the Anderson–Darling goodness of fit test can be used as a criterion for the choice of a parametric distribution for data-rich data sets, because it gives more weight to the tails of the distribution. Results should be discussed in regards to the graphical representation of the species distribution. If the data do not fit any distribution, the left tail of the distribution (the

lowest effect concentrations) should be analysed more carefully. Any choice of a specific distribution function should be clearly explained;

- Estimated parameter, e.g., the concentration corresponding with the point in the species sensitivity distribution (SSD) profile of the chosen toxicity value (e.g., EC_{10}) below which 5% of the species occur may be derived with a 50% confidence interval associated with this concentration, as an intermediate value in the determination of the PNEC;
- Estimation of the PNEC, i.e. the intermediate value may de divided by an appropriate assessment factor, if needed, to reflect the further uncertainties identified. If mesocosm studies are available, they should also be evaluated to decide on the assessment factor;
- Deviations from these recommendations can be made on a case by case basis, through consideration of sensitive endpoints, sensitive species, mode of toxic action and/or knowledge from structure activity considerations;

The PNEC should also be derived, as a comparison, by applying the assessment factor approach on the same database.

ANNEX 2

Toxicokinetic Considerations for the Assessment of Chemicals

Initial considerations

Care should be exercised in predicting metabolic pathways and estimating toxicokinetics of chemicals as these can be complex, especially for larger compounds with multiple reactive groups. Chemical structure (in addition to just information on chemical charges) and chemical concentration can also dictate the metabolic pathways. Failure to appropriately consider this and other critical information may lead to misleading or inaccurate estimations. If predictions are used within the OECD Cooperative Chemicals Assessment Programme, they should be clearly stated as predictions and should be limited to well-established assumptions that are discussed in standard reference materials.

Predictions of toxicokinetic information should be limited to certain cases. For example, some predictions and assumptions may be useful for category approaches that rely on metabolic arguments, especially for chemical classes that have well-known patterns of biotransformation. However, even in these cases, the approaches should be supported with existing data for chemicals within the category.

This document is useful as a repository of toxicokinetic information and experience within the OECD Cooperative Chemicals Assessment Programme and could evolve as more information is obtained. The document can be used to guide assessors to more extensive references on toxicokinetics rather than as a prescriptive guidance document.

This document should not be used as stand alone or prescriptive guidance but rather as a pointer for assessors to more extensive references on toxicokinetics. Given the complexities involved, predictions should be made on a case by case and should be well substantiated.

Background

When revising the OECD guidance for drafting of SIAP's it was agreed (at SIAM 25) that a toxicokinetic assessment should be included when toxicokinetics information is available, although toxicokinetic studies are not part of the SIDS end points. When toxicokinetics information is not available, it may be possible to make some predictions of toxicokinetics on a substance or category by taking account of the available physicochemical and toxicodynamic information.

It is conventional to order a toxicokinetic assessment as follows; Absorption, Distribution, Metabolism and Excretion (ADME). It is not necessary to include these as discrete sub-headings, but it should be possible for the reader to identify the individual processes. In each case, it is not intended to provide a quantitative estimate of these processes, but a more qualitative assessment based on established kinetic and toxicological principles. Robust Study Summary Templates outline reporting formats for studies referred to during an assessment.

The following highlight some brief points that should be taken into consideration when developing a toxicokinetic assessment. However, it is not intended to provide exhaustive guidance and should not be used as stand- alone document. A very limited reference list is included, if further information is required.

Physicochemical Considerations

The information available on the physicochemical properties and chemical structure can be used to make some predictions regarding the ADME of substances. One very important factor relevant for discussion of absorption, distribution as well as excretion is permeability. Permeability of a membrane to a chemical is particularly dependent on molecular size (more than only weight), lipophilicity as well as charge, and also pKa. More information is included in points to consider, see below.

Points to consider

Absorption

Consideration of relevant physico-chemical parameters can inform on the potential to cross biological membranes, ie a log P of around 2-4 suggests that a substance could readily cross biological membranes. Similarly the pKa of ionisable organic molecules will influence whether a molecule can cross a biological membrane. This is because uncharged molecules more readily cross the lipid environment of biological membranes by passive diffusion. For example acetic acid is not ionised at low pH of the stomach, favouring passive diffusion but once in the blood stream it will rapidly ionise and exists as almost exclusively acetate ions. The absorption of molecules with a favourable Log P will be influenced by molecular size, as smaller molecules more readily cross biological membranes. Depending on the chemical in question, consideration of sublingual absorption may be necessary (e.g. H_2O_2) as that route, can allow a proportion of the dose to bypass first-pass metabolism. It should be noted that absorption via the lymphatic system for large molecules should not be excluded.

Molecular weight will also influence uptake, with higher molecular weight substances tending to be less well absorbed, for example waxes will largely transit the GI tract without appreciable uptake.

Toxicodynamic information, such as target organ toxicity distant from the portal of entry can be used as an indication that the substance or a metabolite has been absorbed. If there is absorption via the gastro-intestinal tract, some predictions can be made regarding the inhalation and dermal routes of exposure. Absorption via the respiratory tract is dependent on particle size. In general, uptake via the respiratory tract may be similar to (or less than) absorption via the oral route of exposure. However, it is possible that physico-chemical properties may limit (water solubility) or enhance uptake (volatility) from the respiratory tract. For certain molecules it is possible that they might be internalised by specific transporter systems, for example certain metal ions. It should be borne in mind that the respiratory and intestinal epithelia have evolved to facilitate uptake, whereas the skin provides a barrier function.

Distribution

The distribution of absorbed substances will be particularly influenced by Log P, pKa and molecular size, as the primary means of distribution is via the circulatory system. If the substance is or can be predicted to be soluble in physiological fluids, it will probably be well distributed. Evidence for this may be found from, for example, mouse bone marrow micronucleus studies, if suppression of bone marrow activity is observed this should be regarded as good evidence for wide distribution – if via the oral route of exposure. Substances like some dietary fatty acids are distributed from the intestinal epithelium to the thoracic portal via the lymphatics prior to systemic distribution. Differences observed between single and repeated-dosed studies may provide information on accumulation or enzyme induction. Substances with higher Log P values may distribute preferentially to the more fatty tissues and may, have bioaccumulation potential. Such lipophilic substances may also cross the blood-brain and blood-testis barriers. Similarly, more water soluble molecules will tend to distribute more widely than larger less soluble ones, because of the large

volume of total body water. The degree of protein binding to such as blood albumin will modify the concentration of free compound available to for crossing barriers, undergoing metabolism and induce toxicity.

As with absorption, target organ toxicity away from the portal of entry is an indication that a substance may be widely distributed.

Metabolism

It is possible to make some predictions of likely metabolites, most usefully for organic molecules, based on considerations of chemical structure, physico-chemical properties and any established species differences (usually quantitative) in metabolism. The results of *in vitro* tests can indicate which metabolic pathways are likely to be relevant to detoxication and/or toxicity. Log P will influence metabolism, such as by cytochromes P450. In general substances that are less water soluble make the better substrates and may undergo more extensive biotransformation. This is because the Phase 1 enzymes add or reveal functional groups that make the molecule more water soluble and to facilitate Phase II conjugation reactions. Molecules having undergone Phase I reactions and those with available functional groups, such as -OH or $–NH_2$ tend to undergo Phase II conjugation reactions. Systemic exposure to unchanged parent may be limited if there are hydrolysable functional groups present. It is possible that low capacity phase I reactions (for example those catalysed by 2E1) can become saturated at high substrate concentrations and this may lead to a shift in metabolite profile, or parent to metabolite ratio. For very large chemicals, distribution to fatty tissues is often faster than biotransformation in clearing the compound from the systemic circulation.

Excretion

The aims of this section are to provide some ideas on whether the substance would be excreted unchanged or not, and whether urinary or faecal excretion is more likely.

If it is clear that the substance will (will not) be extensively metabolised, then excretion of unchanged parent is (is not) expected to be limited In considering potential routes of excretion, again Log P will have a significant impact, with the more water soluble substances being excreted via the urine, like polar metabolite, without a requirement for extensive metabolism. Molecular weight is an important determinant in biliary excretion with polar substances of a molecular weight of >350 being more readily excreted via the bile in rats. There is a clear species difference with biliary excretion, as the molecular weight cut off in humans is around 500. As noted above, milk is a route of elimination for some compounds. Excretion via the exhaled air of xenobiotics is largely confined to low molecular weight volatile substances. The induction of pathology within targets such as membranes, barriers, kidneys or the liver can lead to changes to the ADME characteristics of compounds.

Other

It would be useful to provide a qualitative statement on potential exposure of the neonate and/or the developing foetus *in utero*; for example, "Substance x is very lipophilic and excretion of absorbed substance x via the breast milk cannot be excluded", Further information on factors influencing excretion via breast milk can be found in the REACH guidance document on information requirements and the US EPA document "Exploration of Perinatal Pharmacokinetic Issues".

If possible consideration should also be given to toxicokinetics by other relevant routes of exposure. For example, water soluble substances that become systemically available following dermal or inhalation absorption would be expected to be widely distributed.

Information from close structural analogues should also be considered, where appropriate and the utilisation of computational non-testing methods may aid read-across and the filling of data gaps.

References

European Chemicals Agency (ECHA), 2008. Guidance on information requirements and chemical safety assessment. Chapter R.7c: Endpoint specific guidance: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7c_en.pdf?vers=20_08_08 , see R.7.12.